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Chromosome and enzyme diversity in some species of Neanuridae (Collembola)[1]

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With 6 figures

1. Introduction

The biogeography, ecology and cytogenetics of neanurid Collembola are of considerable interest but Cassagnau (1980) stresses the difficulties of working with this group when systematics are mainly based on the structure of the mouth parts. Cassagnau (1980) therefore uses the chaetotaxy of dorsal tubercles, which he suggests is a powerful discriminatory character, to classify all the known genera belonging to the family in a more rational system. However, the systematics of the group still seems to be complicated by the existence, at least in certain genera, of chromosomal polymorphisms with respect to either the shape (Cassagnau, Dallai & Deharveng 1979) or the abundance of heterochromatic material (Cassagnau 1976; Dalens 1979; Dallai 1979). These polymorphisms might well have some adaptive significance, or be involved in speciation processes leading to the differentiation of sibling species, or both.

In order to evaluate the actual significance of such polymorphisms we have started a study of the gene-enzyme systems in some neanurid species using electrophoresis techniques.

It is generally recognized that electrophoresis is a technique providing an extremely valuable tool to investigate microevolutionary processes (Avise 1974; Lewontin 1974; Powell 1975; Ayala 1975; Bullini & Sbordini 1980). Using this method we have been able to demonstrate a remarkable genetic divergence between populations of *Bilobella aurantiaca* as well as of *Thaumanura ruffoi* from different localities which suggests the existence of a differentiation at the species level.

2. Material and methods

The study was carried out on two neanurid species. Populations of *Bilobella aurantiaca*, a widespread species in west Europe, came from the following localities: Siena, Castelporziano (Rome) and Astroni (Naples); the latter is the type locality of the species (Caroli 1910). Populations of *Thaumanura ruffoi*, a species inhabiting cool and damp environments in central Europe and living in several localities along the Appennines (Dallai 1969), came from Montalbuccio (Siena), Barbaione (Siena), a locality only few kilometers from the former, and Astroni (Naples). The second population was used as a control for evaluating the genetic distance between local populations (Fig. 1).

An average of 15 individuals for each population of *Bilobella aurantiaca* were sampled. The proteins scored, each encoded by a single locus, were: PGM (phosphoglucomutase), PHI (phosphoesosoisomerase), APH (alkaline phosphatase), HK (hexokinase).

An average of 30 individuals were sampled for each population of *Thaumanura ruffoi*. For this species the proteins scored were as follows: ADKIN (adenylate kinase), APH (alkaline phosphatase), CA (carbonic anhydrase), EST (esterase), G6PG (glucose-6-phosphate dehydrogenase,) HK (hexo-

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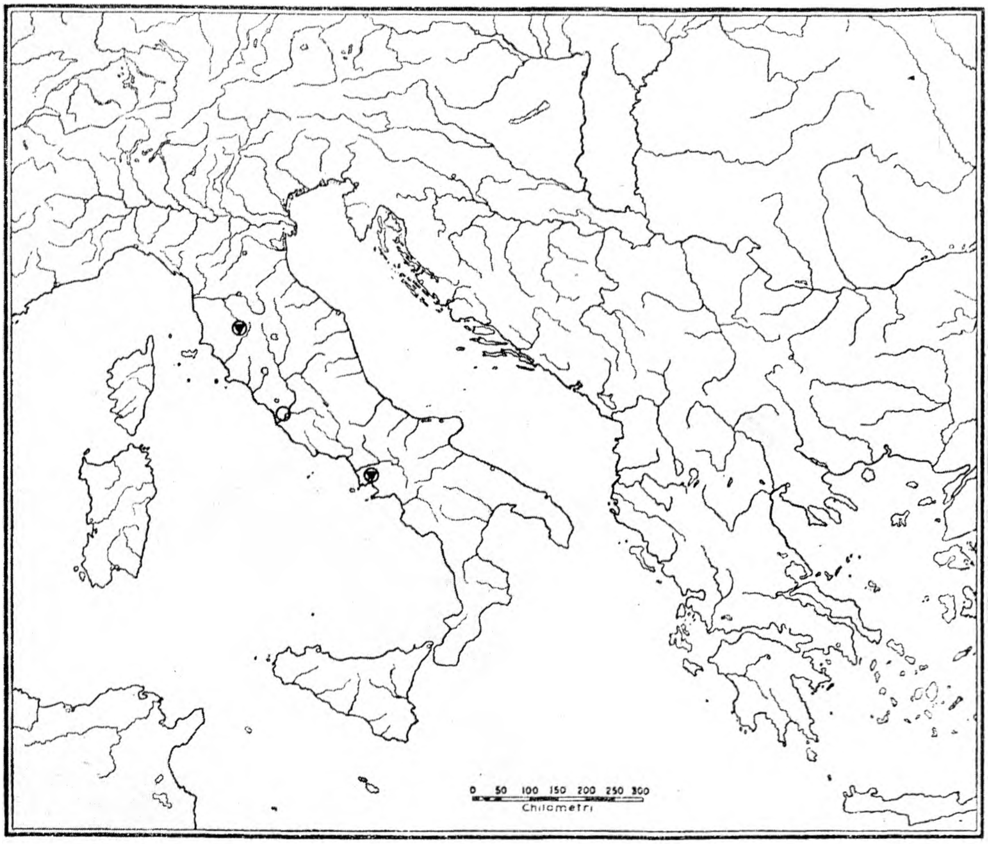

Fig. 1. Sampling localities for the populations assayed for gene-enzyme electrophoretic variations. ○ *Bilobella aurantica* [Siena, Rome, Naples], ▼ *Thaumanura ruffoi* [Siena and Naples].

kinase), LAP (leucine aminopeptidase); MDH (malate dehydrogenase), MPI (mannose phosphate isomerase), 6-PGD (6-phosphogluconatedehydrogenase), PHI (phosphoesosoisomerase), PGM (phosphoglucomutase). Overall, a set was studied of 15 loci encoding for these proteins.

Alleles are designated by a number indicating the mobility of their products relative to the most common allele which is given a mobility of 100.

Polytene nuclei of the different populations of *Bilobella aurantiaca* and *Thaumanura ruffoi* were prepared from dissected salivary glands of animals previously fixed in Carnoy. The staining procedure is that described by Cassagnau (1968). Mitotic chromosomes of *Thaumanura* were obtained from intestinal and embryonic mitosis; testicular meiosis was also observed.

3. Geographic distribution

Bilobella aurantiaca is a species common in the whole mediterranean area (Fig. 2). According to Cassagnau (1976) it belongs to a neanurid group of Indian-African origin which reached the Mediterranean region at the age of the paleogeographic movements of the Earth's surface occurring in this area. Usually animals live under barks of fallen evergreen oak, but they may also be found in different habitats. The animals are commonly yellow-orange, but different body pigmentations are often seen, evidently unrelated to specific variation.

Thaumanura ruffoi belongs to a genus distributed in central and west Europe. The species in Italy is known from the Venetian pre-Alps, Appennines and mountains in Sicily (Dallai 1969, 1970, 1971 and unpublished; Dallai & Martinozzi 1980). Recently Deharveng (1979) found the species on Maures mountains which represent the west limit of the distribution (Fig. 3). It is difficult to collect large samples of this species because of the dark colour, limited mobility and its occurrence in cryptic microhabitats within fallen tree barks, particularly the barks of evergreen oak.

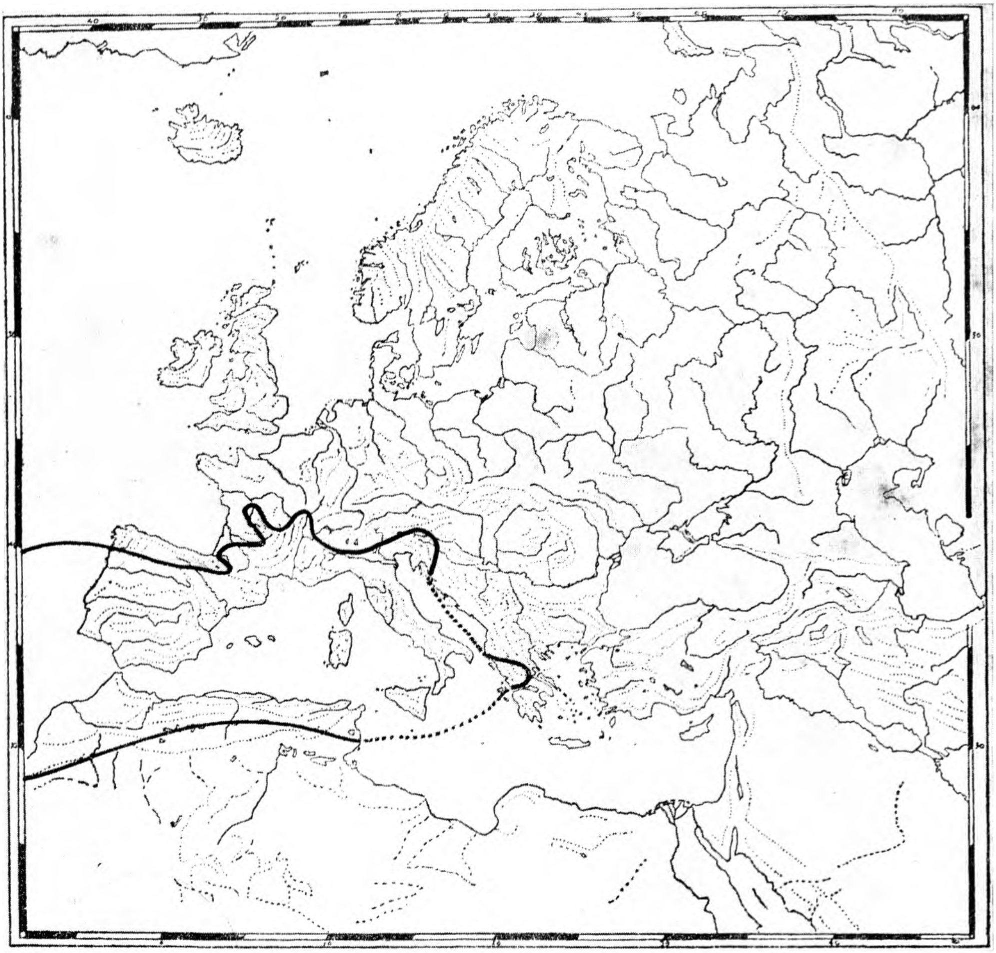

Fig. 2. Geographic distribution of *Bilobella aurantiaca* (from Cassagnau 1976).

4. Results and discussion

Bilobella aurantiaca has a diploid number 2 n = 14. The mitotic chromosomes were very small and it is practically impossible to detect differences between the examined populations. Polytene chromosomes, on the contrary, show clear differences mainly in their α-heterochromatin (Dallai 1979). The amount of heterochromatin seemed to be less in the Naples' population than in the Rome and Siena populations (Fig. 4). In addition, while chromosomes n. 6 and n. 7 had almost the same shape in the Rome and Siena populations, they show a different aspect in the Neapolitan population (Fig. 5).

The results obtained from electrophoretic studies of gene-enzyme system in *Bilobella aurantiaca* are reported in the Table 1.

Two loci were found to be monomorphic for all the three populations of *Bilobella aurantiaca:* APH and HK. APH was fixed for the same allele in every population, while at HK locus the Neapolitan population was fixed for an alternative allele. The remaining three loci (PGM, PHI, LAP), in which the commonest variant had a frequency less than 0.99, were polymorphic for two or more alleles (Table 1).

Starting from allelic frequencies the genetic identities and the genetic distances calculated according to the Nei's method for the three different populations of *Bilobella aurantiaca* were as in Table 2.

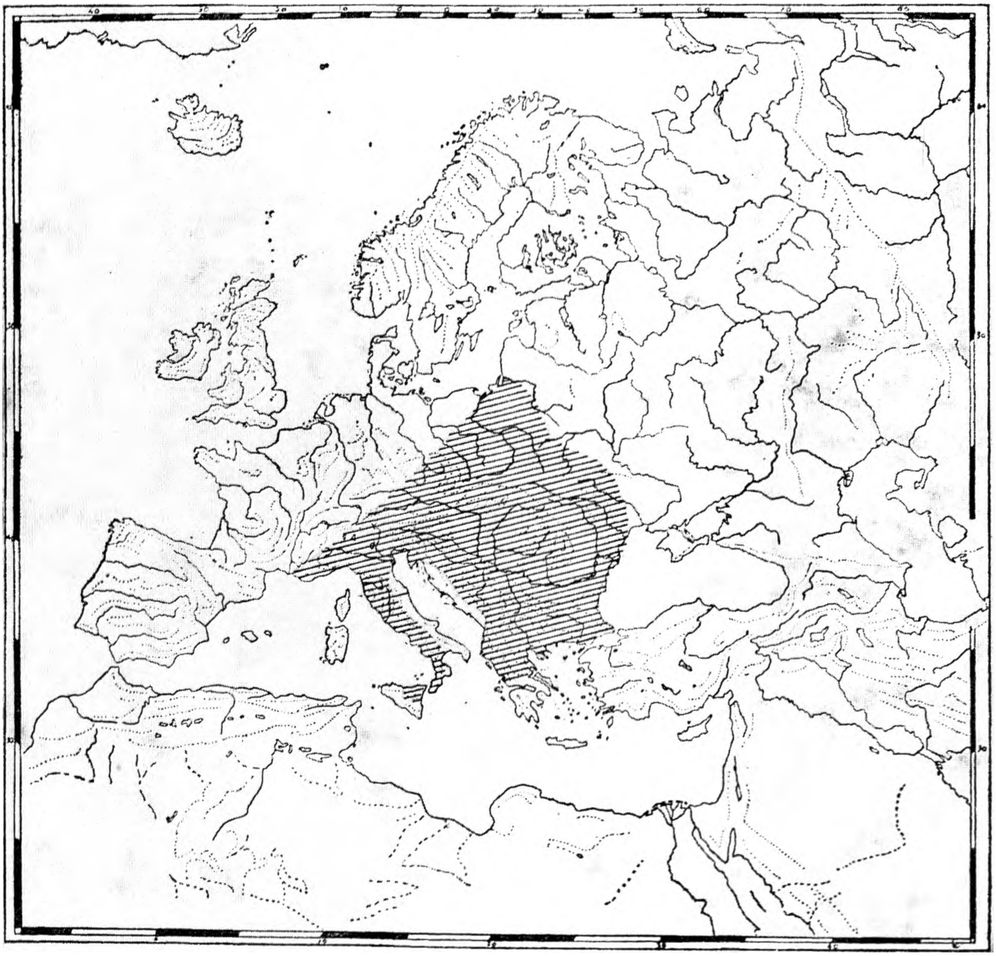

Fig. 3. Geographic distribution of the genus *Thaumanura*.

Genetic distance values between the Rome and Siena populations are low and comparable to those found between local conspecific populations. On the contrary the values between Neapolitan population and/or Rome and Siena populations are fairly high, comparable to the data obtained between sibling species or even between species morphologically well differentiated (Ayala 1975; Avise 1976).

The average value of heterozygosity per population is quite high so that it seems unlikely that a recent founder effect has played some role (Table 3).

Results from caryological studies agree with those obtained with gene-enzyme system method. The existence of differently shaped chromosomes and of a smaller amount of the α-heterochromatin in the southern populations (Dallai 1979), could be taken as an indication of a different origin of these populations compared with those of central Italy. The results of the gene-enzyme study, even if preliminary, seem to support this hypothesis.

As to *Thaumanura ruffoi*, because of the small size of chromosomes, we were able only to establish that in every population the diploid chromosomic number was $2n = 16$ ($n = 8$) (Fig. 6). This number was seen in embryonic mitosis and in preparations of testes which showed meiotic divisions. Mitosis were also observed in intestinal cells, but often these nuclei were polyploid. Polytene chromosomes in *Thaumanura ruffoi* were very difficult to study because of the low degree of polyteny of the chromosomes which appeared very thin and

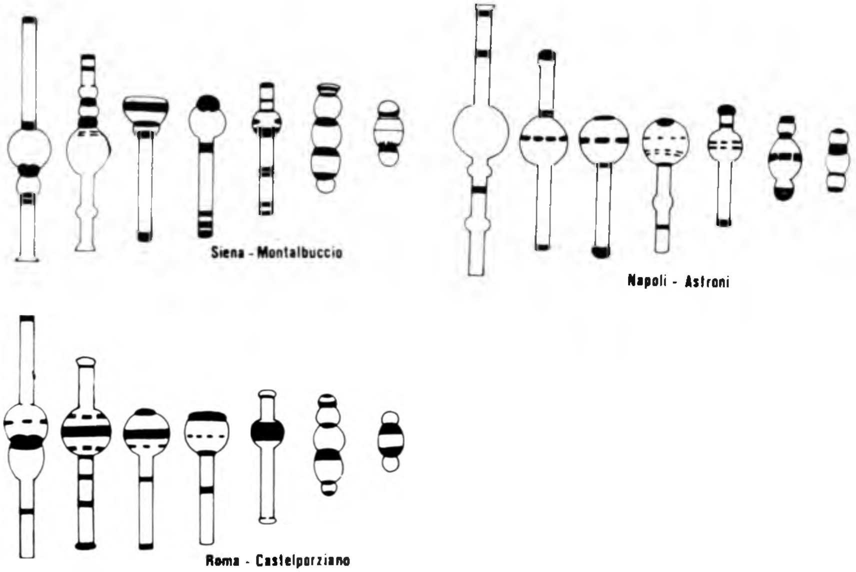

Fig. 4. Schematic drawing of the karyotypes of the three different populations of *Bilobella aurantiaca* examined, showing the α-heterochromatin distribution (from Dallai 1979).

Table 1. Allele frequencies observed in the different populations of *Bilobella aurantiaca*

Locus	alleles	Rome	Siena	Naples
Pgm	N	28	20	24
	105	—	—	.542
	102	.357	.750	—
	100	.643	.250	.458
	He	.476	.395	.518
Phi	N	28	28	26
	108	—	—	.154
	105	.179	.107	.154
	100	.821	.857	.269
	95	—	.036	.423
	He	.304	.263	.729
Lap	N	16	20	12
	100	.750	.750	.833
	95	.250	.250	.167
	He	.409	.395	.303
Aph	N	24	32	26
	100	1.0	1.0	1.0
Hk	N	10	12	8
	105	—	—	1.0
	100	1.0	1.0	—

long and extremely interwined. As a consequence it was difficult to detect differences between the populations.

The genetic situation in *Thaumanura ruffoi* was more carefully examined with the gene-enzyme systems. This species was monomorphic at six of the fifteen analyzed loci: CA-1, EST-1, G6PD, MDH, 6-PGD, PHI, the remaining nine loci were polymorphic for two or more alleles (Table 4). In a few cases single populations were monomorphic at the same single loci. For instance, in addition to the above mentioned loci, the Neapolitan population

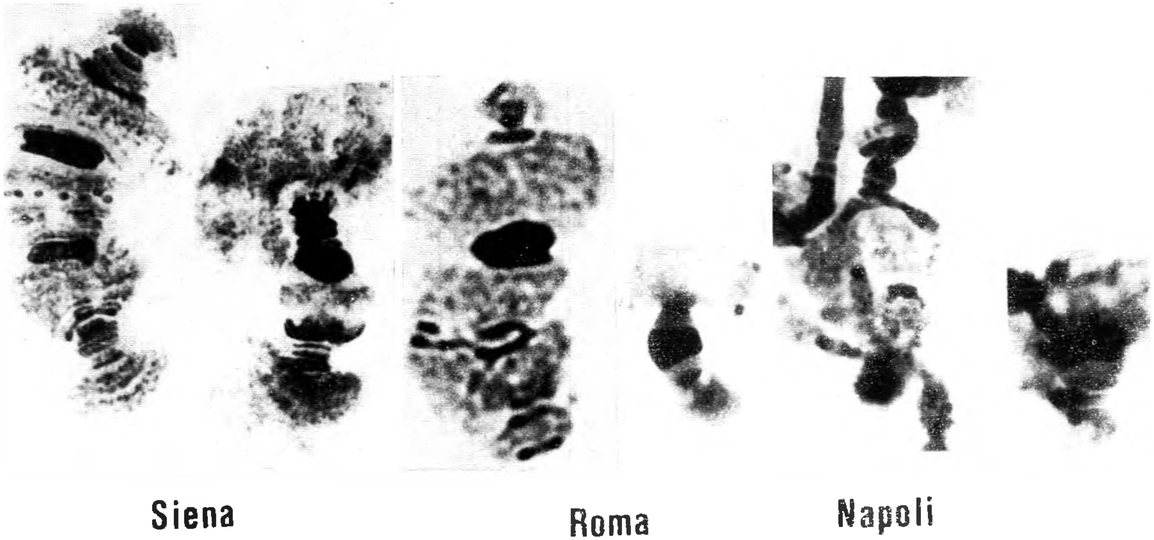

Fig. 5. The morphology of chromosomes n. 6 and n. 7 in the three different populations of *Bilobella aurantiaca* examined (from DALLAI 1979, modified).

Table 2. Genetic identities (above) and genetic distance (below) in the populations of *Bilobella aurantiaca*

	Rome	Siena
Siena	.960	—
	.041	—
Naples	.598	.544
	.514	.600

Table 3. Estimates of genetic variability in three populations of *Bilobella aurantiaca*

Populations	n° loci	He	A	P
Siena	5	.210	1.8	.6
Rome	5	.238	1.6	.6
Naples	5	.310	2.2	.6

(He) average frequency of heterozygous individuals per population expected at Hardy-Weinberg equilibrium; (A) average number of electrophoretically detectable alleles per locus; (P) proportion of polymorphic loci.

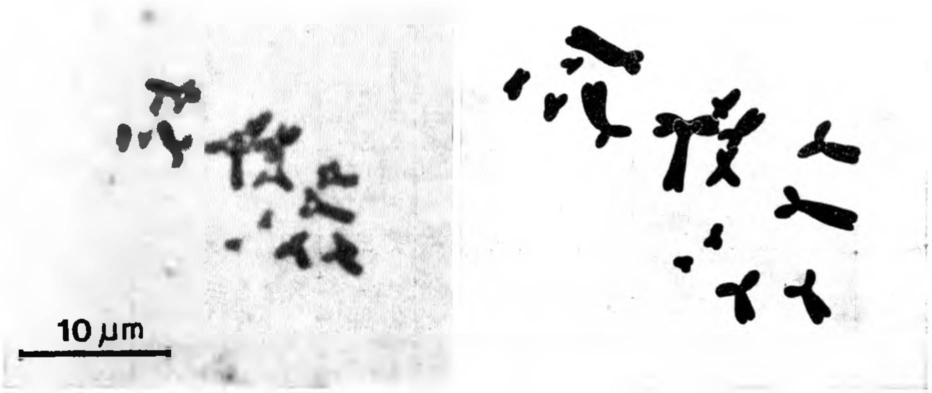

Fig. 6. *Thaumanura ruffoi.* Left-side, embryonic mitosis showing the diploid number 2n = 16 right-side, the reconstruction of the same mitosis.

was also monomorphic at the following loci: APH, EST-2, EST-3, MPI, PGM; Siena population at: HK and LAP; Barbaione population at: ADKIN, LAP and PGM. All three populations were identical at the EST-1 locus; in addition they showed similar frequencies at the loci: ADKIN, APH, CA-2, EST-2, EST-3. Eight loci: CA-1, G6PD, LAP, MDH, MPI, 6-PGD, PHI and PGM were discriminating between Siena and Naples populations as they were fixed for alternative alleles. On the other hand, these eight loci were fixed for the same alleles or showed similar allele frequencies in Siena and Barbaione populations which are spatially very close one to another.

Starting from allelic frequencies the genetic identities and the genetic distances calculated according to the Nei's method for three populations of *Thaumanura ruffoi* were as in Table 5.

The low genetic distance found between Siena and Barbaione is consistent with the vicinity of the two populations, the value well fitting with most date, reported between local populations. The situation between Siena and/or Barbaione and Naples clearly shows a genetic differentiation which is comparable to that usually reported between well differentiated species where evidence of reproductive isolation is available (Ayala 1975; Avise 1976; Sbordoni 1982).

Table 4. Allele frequencies observed in different populations of *Thaumanura ruffoi*

Locus	alleles	SIENA	BARBAIONE (Siena)	NAPLES
Adkin	N	92	12	62
	103	.043		
	100	.957	1.0	.968
	97			.032
	He	.084		.063
Aph	N	78	10	62
	102	.102		
	100	.885	.90	1.0
	98	.013	.10	
	He	.209	.20	
Ca-1	N	42	12	28
	100	1.0	1.0	
	90			1.0
Ca-2	N	42	12	34
	103	.238	.416	.088
	100	.762	.584	.912
	He	.371	.530	.166
Est-1	N	8	12	28
	100	1.0	1.0	1.0
Est-2	N	8	12	28
	100	.625	.667	1.0
	98		.333	
	97	.375		
	He	.536	.484	
Est-3	N	44	12	28
	105	.045	.167	
	100	.682	.750	1.0
	95	.273	.083	
	He	.469	.439	
G6pd	N	94	12	62
	100	1.0	1.0	
	99			1.0
Hk	N	82	12	62
	104		.250	
	103			.500 (1.0)
	100	1.0	.750	.500
	He		.409	
Lap	N	82	12	62
	102			.500 (1.0)
	100	1.0	1.0	.500
Mdh	N	94	12	62
	105			1.0
	100	1.0	1.0	
Mpi	N	60	12	62
	100	.900	.917	
	97	.100	.083	
	94			1.0
	He	.183	.167	
6 Pgd	N	94	12	60
	105			1.0
	100	1.0	1.0	
Phi	N	56	12	60
	110			1.0
	100	1.0	1.0	
Pgm	N	82	12	60
	100	.597	1.0	
	97	.403		
	90			1.0
	He	.487		

Table 5. Genetic identities (above) and genetic distance (below) in the populations of *Thaumanura ruffoi*

	Siena	Barbaione (Siena)
Barbaione (Siena)	.967 .0033	— —
Naples	.440 .821	.429 .847

Table 6. Estimates of genetic variability in the three populations of *Thaumanura ruffoi*

Populations	n° loci	He	A	P
Siena	15	.16	1.60	.47
Barbaione (Siena)	15	.15	1.47	.40
Naples	15	.015	1.13	.13

(He) average frequency of heterozygous individuals per population expected at Hardy-Weinberg equilibrium; (A) average number of electrophoretically detectable alleles per locus; (P) proportion of polymorphic loci.

The values in Table 6 account for a high degree of genetic variability in the two Sienese populations and by contrast for a very low degree of variability in the population of Naples.

These data might be interpreted as the result of the initial founder effect in a recent colonization of the Neapolitan region starting from populations living in the neighbouring mountains. The Neapolitan population in fact lives just in the crater of the Astroni vulcan. This volcano has been subject to intense activity up to about 11,000 years ago (Alessio *et al.* 1973; Bruni, Sbrana & Silvano 1982).

Both population size and the recent history of the Astroni population could account for its low heterozygosity. Such a hypothesis could be tested by studies on other populations in the neighbourhood of Naples designed to detect differences in the genetic variability.

Is the high value of genetic distance between the populations of Siena and Naples sufficient to retain them as two good species even if morphologically undifferentiated?

Although no evidence of reproductive isolation is presently available on *Bilobella aurantiaca* and *Thaumanura ruffoi*, an increasingly large set of data obtained from a wide spectrum of taxa indicates that two populations may reach reproductive isolation and complete the speciation process even at lower values of genetic distance (Ayala 1975; Sbordoni 1982).

Experiments on crosses between the different populations would be of interest; however this might be difficult because of the type of reproduction habits in Collembola. Direct insemination does not occur; instead the males deposit spermatophores on the ground which are picked up subsequently by the females (Shaller 1979).

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Discussion

Questions de P. Cassagnau, Toulouse: 1. Existe-t-il des différences dans la teneur en hétérochromatine entre les populations de *Thaumanura* dont la distance génétique est forte?
2. Peut-on comparer la distance génétique entre deux populations dans des groupes taxonomiques éloignés?

Réponses de R. Dallai: 1. En ce qui concerne l'hétérochromatine il est difficile de dire s'il y a des différences; les chromosomes géants de *Thaumanura* ne sont pas bien étalés et quand ils sont superposés il faut faire attention de ne pas prendre des bandes pour des plaques d'hétérochromatine.
2. Pour la deuxième question il faut rappeler que le système gène-enzymes n'est pas applicable à un niveau supérieur à celui de l'espèce ou même de genres voisins. L'électrophorèse donne seulement des indications sur les substitutions des aminoacides qui modifient la mobilité électrophorétique, mais elle n'est pas capable de nous dire combien de substitutions ont été réalisées pendant la cladogenèse.

Synopsis: *Original scientific paper*

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In recent years polytene chromosomal studies on different neanurid Collembola have revealed a great variability within a single species in heterochromatin density and chromosome shape. This paper reports analyses of chromosome and enzyme polymorphisms of some populations to obtain information of the genetic distance between them.

Three populations of *Bilobella aurantiaca* from Siena, Rome and Naples are distinguishable on the basis of the different concentration of heterochromatin which is less abundant in the caryotype of the Naples population compared with the other two populations. The genetic divergence values confirm the similarity between the Siena and Rome populations and their differentiation from that of Naples.

Two populations of *Thaumanura ruffoi*, morphologically and cariologically undifferentiated, from Siena and Naples, tested with the same technique, reveal a high value of genetic separation.

Our data, if compared with genetic distances found for various levels of evolutionary divergence in other insects, suggest the existence, in both the examples examined, of two distinct specific taxa, even though a reproductive isolation has still to be demonstrated.

Key words: Polytene chromosomes, enzymatic polymorphism, Collembola, speciation.